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Pharmacokinetics and biochemical efficacy after single and multiple oral administration of losartan, an orally active nonpeptide angiotensin II receptor antagonist, in humans

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1 The pharmacokinetics and biochemical efficacy of losartan, an orally active nonpeptide angiotensin II (AII) receptor antagonist, were evaluated in healthy male volunteers after single and multiple oral administration.
2 Plasma and urinary concentrations of losartan and its active metabolite, E-3174, were determined by a specific high performance liquid chromatographic (h.p.l.c.) method.
3 Plasma concentrations of losartan were proportional to dose over the range of 25 to 200 mg and the terminal half-lives ($t_{1/2,z}$) ranged from 1.5 to 2.5 h. The mean values of C_{max} and AUC0-∞ increased in a dose-dependent manner.
4 Plasma concentrations of E-3174 were higher than those of losartan at all dose levels. The values of C_{max} and AUC0-∞ for E-3174 were approximately 2 and 5–8 times higher than those for losartan, respectively. Also the value of $t_{1/2,z}$ was 2 times longer than that of losartan.
5 After multiple dosing for 7 days, the pharmacokinetics of losartan and E-3174 each did not change significantly between day 1 and day 7.
6 Plasma renin activity (PRA) and plasma concentrations of AII increased markedly at all dose levels. Plasma aldosterone levels were slightly reduced, but a similar decrease was also observed with placebo.
7 No clinically significant adverse reaction was observed in any of the volunteers during either study. Blood counts, routine laboratory tests, urine analyses, and electrocardiograms were also not modified by losartan.
8 Losartan appears to be a potent orally active angiotensin II antagonist with a relatively long duration of action.

Keywords angiotensin II antagonist losartan pharmacokinetics human volunteers

Introduction

Losartan, the potassium salt of 2-*n*-butyl-4-chloro-1-[2′-(tetrazol-5-yl)-1, 1′-biphenyl-4-ylmethyl]-1H-imidazole-5-methanol, has been synthesized by E.I. du Pont de Nemours and Company (Wilmington, DE, USA) as a novel orally active nonpeptide angiotensin II (AII) receptor antagonist (Timmermans *et al.*, 1990; Wong *et al.*, 1990a). *In vitro* and *in vivo* studies showed that losartan is much more selective for the AII-1 site than the AII-2 (Chiu *et al.*, 1990a) and that it is devoid of partial agonist activity (Wong *et al.*, 1990a). Losartan is distinguished by its competitive mode of action, and these characteristics are in sharp contrast with that of peptide antagonists such as saralasin and [Sar_1Ile_8]-AII (Chiu *et al.*, 1990b; Freer *et al.*, 1980). Therefore, losartan is classified as the first generation of nonpeptide AII blockers which shows specificity and high affinity for type I angiotensin receptors (Chiu *et al.*, 1989). Given by either oral or intravenous administration, losartan decreased blood pressure significantly in conscious renal-ligated rats (Wong *et al.*, 1990b) and in conscious

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spontaneously hypertensive rats (Wong *et al.*, 1990c). Thus, losartan is anticipated to have therapeutic utility for the treatment of hypertension.

Christen *et al.* (1991a,b) assessed the inhibitory effect of losartan on the pressor action of exogenous angiotensin I and II in healthy volunteers, and they have suggested that losartan is a potent and long-acting antagonist of AII in humans. However, they have not reported the pharmacokinetics of losartan and its active metabolite, E-3174 (Wong *et al.*, 1990d). It is important to obtain preliminary pharmacokinetic and efficacy data for the drug from healthy volunteers before initiation of further clinical studies.

The objective of this study was to investigate the pharmacokinetics and biochemical efficacy of losartan after single and multiple oral dosings in healthy male volunteers.

Methods

Drugs

Losartan; E-3174, 2-*n*-butyl-4-chloro-1-[2′-(tetrazol-5-yl)-1,1′-biphenyl-4-ylmethyl]-1H-imidazole-5-carboxylic acid and Dup 167, 2-*n*-propyl-4-chloro-1-[2′-(tetrazol-5-yl)-1, 1′-biphenyl-4-ylmethyl]-1H-imidazole-5-aldehyde were synthesized at E.I. du Pont de Nemours and Company (Wilmington, DE, USA).

Subjects

A total of 33 healthy male subjects, aged 23–48 years (mean 34) and weight 48.8–84.6 kg (mean 64.3), participated in the two consecutive studies. All subjects had a medical history taken, completed a physical examination, and underwent standard clinical laboratory testing prior to the study. Subjects were excluded from the study if they had taken any drug within 2 weeks of the initiation of the study. All subjects gave written informed consent for the study which was approved by the Hospital Research and Ethics Committee of Hamamatsu University School of Medicine. Twenty-four subjects were randomly assigned to four panels of six subjects each to receive rising single doses of 25, 50, 100 and 200 mg. The multiple-dose study was a double-blind placebo-controlled, randomized study. Nine subjects were randomly assigned to receive 100 mg ($n = 6$) or placebo ($n = 3$) once a day for 7 days.

Study design

In the single-dose study, dosing with the drug (25, 50, 100 and 200 mg, as losartan capsules) was preceded by an overnight fast, and no food was allowed until 4 h post-dosing. In the multiple-dose study, on days 1 and 7, dosing with the drug (100 mg losartan capsule or placebo) was preceded by an overnight fast. On other days, the drug was administered 30 min after breakfast.

Blood sampling was done before and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24 and 30 h after the single dosing. In the multiple-dose study, the samples were collected before and at 4 and 24 h after each dosing. On days 1 and 7, additional samples were taken at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 and 30 h after dosing. All blood samples were immediately centrifuged and the plasma was separated and frozen at −80° C. Pooled urine was collected from each subject over 24 h after each dosing, the volume was measured and an aliquot kept frozen at −80° C for analysis.

Analytical procedure

Concentrations of unchanged losartan and its active metabolite, E-3174, in both plasma and urine were determined using a high performance liquid chromatographic (h.p.l.c.) method. Briefly, plasma or urine, to which was added an appropriate quantity of internal standard, Dup 167, was buffered with 0.1 M citrate buffer (pH 2.8). The sample was subjected onto a solid-phase extraction column (Bond Elut C_8 or Bond Elut NH_2 and C_{18}) (Analytichem, Int.) and eluted with an appropriate concentration of methanol. After evaporation of eluate, the residue was re-dissolved with 50% ethanol in water, and then injected (25 or 50 μl) into an h.p.l.c. system consisting of a New Guard RP-18 pre-column packed with ODS (3.2 × 15 mm, Biosystem Inc.) and a Shiseido Capcell Pak C_{18}-ODS analytical column (4.6 × 250 mm, Shiseido Inc.). Elution was with two steps gradient from 35 to 50% (0–10 min) and from 50–65% (10–20 min) acetonitrile in 0.025 M phosphate buffer (pH 2.8), at a flow rate of 1 ml min^{-1}. The eluate was monitored with a u.v. detector at 254 nm. There was no interference by endogenous substances. The quantitative limits of both losartan (as a potassium salt) and E-3174 (as a free acid) were 10 ng ml^{-1} in plasma and 20 ng ml^{-1} in urine.

Biochemical efficacy was evaluated by measuring plasma renin activity (PRA) (Poulsen & Jorgensen, 1974), and plama angiotensin II (AII) (Nussberger *et al.*, 1988) and aldosterone levels (Nussberger *et al.*, 1984) using a radio-immunoassay (r.i.a.). Further, in order to quantitate the hypotensive effect of the drug, blood pressure in a supine position (systolic, diastolic and mean) and heart rate were measured at the forearm using an automatic blood-pressure recorder (Nihon Kohrin, BP-1001).

Data analysis

The terminal elimination rate constant (λ_z) was determined by linear regression of data in the log-linear post-absorptive phase (Gibaldi & Perrier, 1982). Terminal half-life ($t_{½,z}$) was calculated as follows: $t_{½,z} = 0.693/\lambda_z$. Estimation of the area under the plasma concentration-time curve from zero time to infinity (AUC0-∞) was carried out in two steps (AUC0-∞ = AUC0-t + AUCt-∞). The area under the curve from zero to the last time point (AUC0-t) was calculated by means of the linear trapezoidal rule. The area under the curve from the last time point to infinity (AUCt-∞) was estimated as follows: AUCt-∞ = Cp/λ_z, where Cp is the last measured plasma concentration. Apparent renal clearance (CLr0-24) was calculated by dividing the urinary amounts excreted within 24 h by AUC0-24. Both the time to reach peak plasma concentration of the drug (t_{max}), and the corresponding plasma concentration

(C_{max}) were determined directly from the concentration-time curves. Results were given as mean ± s.d. Statistical analysis was by Student's *t*-test, the minimum level of significance being set at $P < 0.05$.

Results

Pharmacokinetics

The mean plasma concentrations of losartan and its active metabolite, E-3174, after single dosing of the drug are presented in Figure 1, and the mean pharmacokinetic parameters observed are summarized in Table 1. The mean values of t_{max} for losartan at all dose levels were about 1 h, and the values of $t_{½,z}$ were about 2 h. The mean values of AUC0-∞ and C_{max} increased in a dose-dependent manner. The values of t_{max} and $t_{½,z}$ for losartan after 50, 100 and 200 mg dosing were not significantly different from the corresponding values after 25 mg dosing.

On the other hand, the mean values of t_{max} for E-3174 were 2.0–3.7 h. The $t_{½,z}$ values were about two times longer than those obtained for losartan. The C_{max} values for E-3174 were about two times higher than

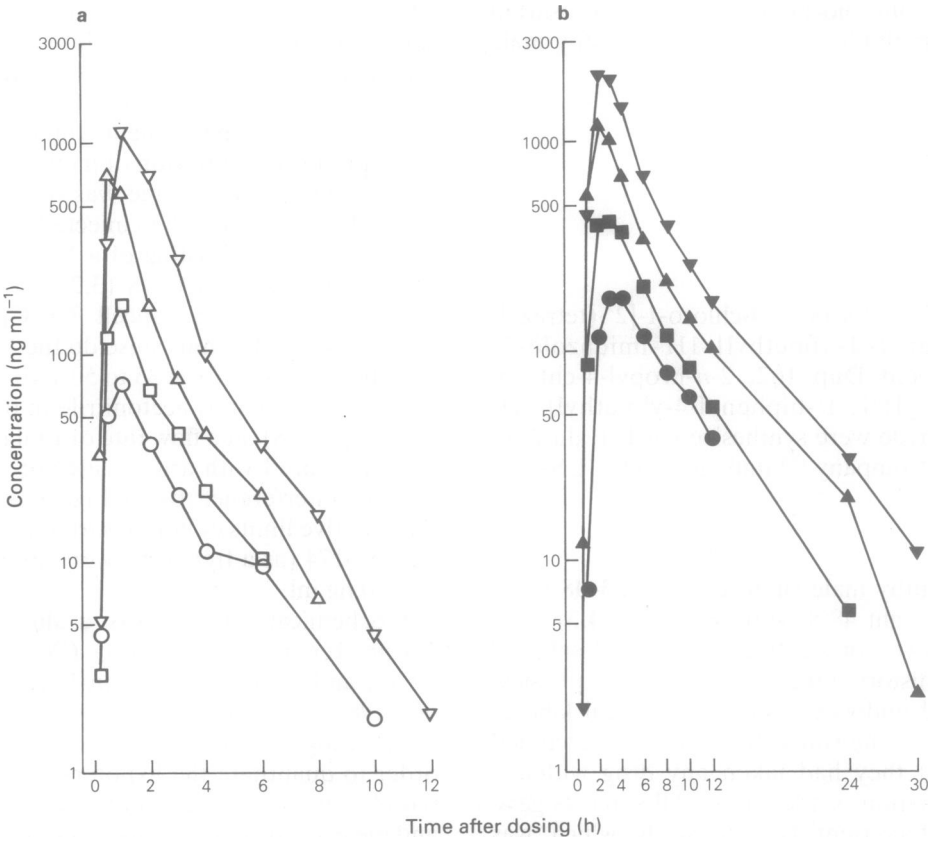

Figure 1 Mean plasma concentrations of losartan (a) and E-3174 (b) after single oral administration of losartan in man ($n = 6$). 25 mg (○, ●), 50 mg (□, ■), 100 mg (△, ▲) and 200 mg (▽, ▼).

Table 1 Pharmacokinetic parameters of losartan and E-3174 in man after single oral administration

	Dose (mg)			
	25	*50*	*100*	*200*
Losartan				
C_{max} (ng ml^{-1})	84.5 ± 40.9	197.6 ± 108.5	800.5 ± 330.9	1394.9 ± 513.1
t_{max} (h)	0.8 ± 0.3	1.3 ± 0.9	0.7 ± 0.3	1.3 ± 0.5
AUC0-∞ (ng ml^{-1} h)	201.3 ± 79.6	354.2 ± 128.8	1069.1 ± 392.6	2231.2 ± 1065.2
$t_{½,z}$ (h)	2.48 ± 1.54	1.69 ± 0.75	1.51 ± 0.41	1.79 ± 0.19
Xu0-24 (% of dose)	3.6 ± 0.9	3.2 ± 0.9	4.1 ± 1.1	3.7 ± 1.6
CLr0-24 (l h^{-1})	6.5 ± 2.5	5.6 ± 2.2	4.1 ± 0.7	3.7 ± 1.8
E-3174				
C_{max} (ng ml^{-1})	188.9 ± 47.3	462.5 ± 161.0	1210.8 ± 271.4	2219.0 ± 484.9
t_{max} (h)	3.7 ± 0.5	3.0 ± 0.6	2.0 ± 0.6	2.5 ± 0.5
AUC0-∞ (ng ml^{-1} h)	1348.9 ± 203.6	2653.3 ± 656.0	5958.2 ± 1192.2	10861.4 ± 3316.5
$t_{½,z}$ (h)	3.80 ± 0.50	3.75 ± 0.70	4.17 ± 0.48	4.37 ± 0.36
Xu0-24 (% of dose)	7.9 ± 1.7	6.9 ± 1.6	7.0 ± 1.4	6.0 ± 1.5
CLr0-24 (l h^{-1})	1.8 ± 0.3	1.5 ± 0.5	1.2 ± 0.3	1.2 ± 0.4

Each value represents the mean ± s.d. of six subjects.

those for losartan. The AUC0-∞ and C_{max} also increased in a dose-dependent manner, and the values of AUC were approximately 5–8 times higher than those obtained for losartan. The each value of mean urinary recovery (Xu0-24) and apparent renal clearance (CLr0-24) for either losartan or E-3174 were not significantly different amoung four doses.

In the multiple-dose study, the dosage level of 100 mg once a day for 7 days was used. The mean plasma concentrations of losartan and E-3174 during and after the multiple dosing are shown in Figure 2, and the mean pharmacokinetic parameters on days 1 and 7 are summarized in Table 2. Each value of C_{max} and AUC0-24 for either losartan or E-3174 was not significantly dif-

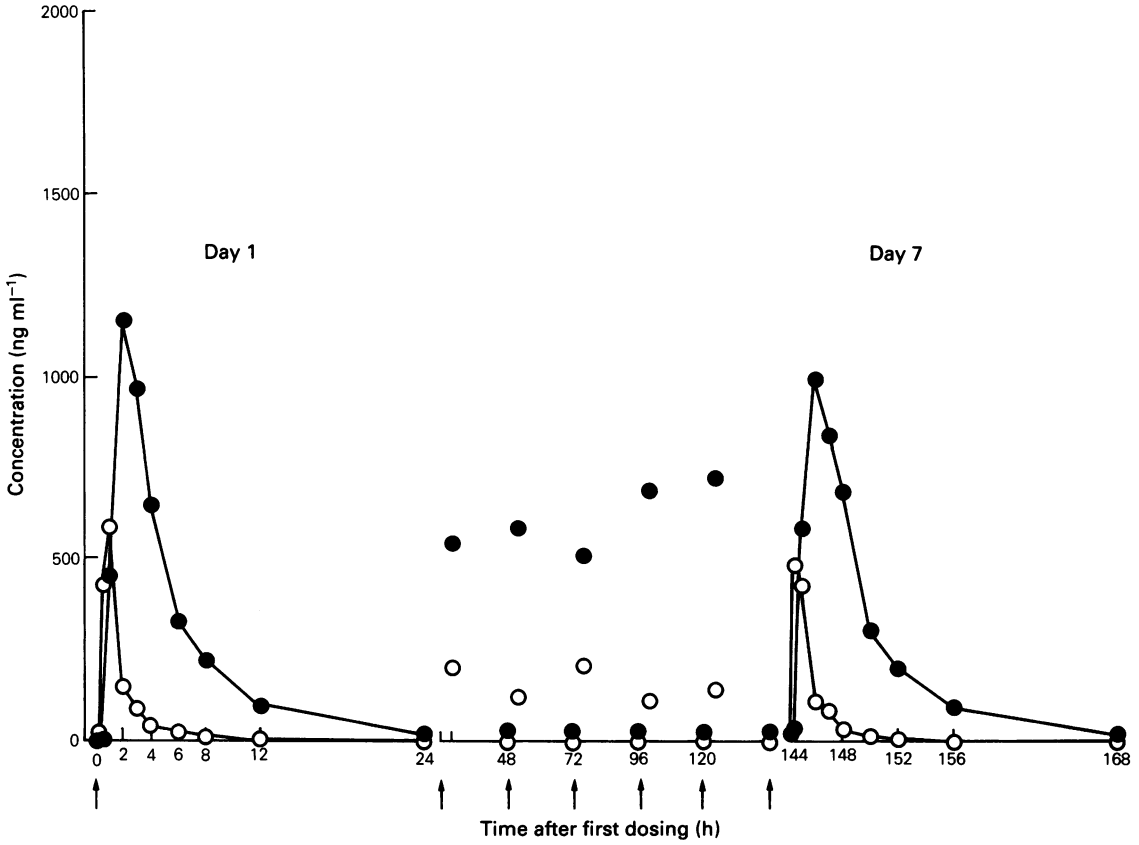

Figure 2 Mean plasma concentrations of losartan (○) and E-3174 (●) during and after multiple oral administration of losartan (100 mg once a day) for 7 days, (n = 6). ↑ : dosing.

Table 2 Pharmacokinetic parameters of losartan and E-3174 in man after multiple oral administration (100 mg once a day) for 7 days

	Day 1	*Day 7*	
Losartan			
C_{max} (ng ml^{-1})	639.8 ± 251.7	655.0 ± 381.4	(NS)
t_{max} (h)	0.8 ± 0.3	0.8 ± 0.3	(NS)
AUC0-∞ (ng ml^{-1} h)	995.3 ± 232.1	806.1 ± 207.9	(NS)
			($P < 0.05$)*
AUC0-24 (ng ml^{-1} h)	952.6 ± 234.7	773.9 ± 202.4	(NS)
$t_{1/2,z}$ (h)	2.27 ± 0.85	1.62 ± 0.80	(NS)
Xu0-24 (% of dose)	4.7 ± 1.5	3.9 ± 0.9	(NS)
CLr0-24 (l h^{-1})	4.9 ± 1.0	5.0 ± 0.6	(NS)
E-3174			
C_{max} (ng ml^{-1})	1227.7 ± 400.3	1074.3 ± 344.8	(NS)
t_{max} (h)	2.3 ± 0.5	2.3 ± 0.5	(NS)
AUC0-∞ (ng ml^{-1} h)	5706.5 ± 1580.6	5508.8 ± 1444.4	(NS)
			(NS)*
AUC0-24 (ng ml^{-1} h)	5535.8 ± 1591.2	5290.7 ± 1443.3	(NS)
$t_{1/2,z}$ (h)	4.54 ± 0.99	4.99 ± 0.98	(NS)
Xu0-24 (% of dose)	6.5 ± 1.8	6.1 ± 1.7	(NS)
CLr0-24 (l h^{-1})	1.2 ± 0.3	1.2 ± 0.3	(NS)

Each value represents the mean ± s.d. of six subjects.
NS: not significant between day 1 and day 7 ($P < 0.05$).
* Difference between AUC0–∞ day 1 compared with AUC0-24 day 7.

ferent between day 1 and day 7. However, in the case of losartan, a statistical difference was found when comparing the value of AUC0-∞ on day 1 with that of AUC0-24 on day 7. The value of AUC0-24/AUC0-24 (day 7/day 1) for losartan was 0.8. Each value of t_{max} and $t_{1/2,z}$ for either losartan or E-3174 did not change significantly during and after the multiple dosing. All plasma concentrations of unchanged losartan at 24 h after every dosing were under the quantitation limits of the h.p.l.c. assay. Each value of Xu0-24 and CLr0-24 for either losartan or E-3174 did not change significantly between day 1 and day 7. There were no significant changes in the urinary excretion during and after the multiple dosing.

Biochemical efficacy

No clinically significant adverse effects were observed in any of the volunteers during either study. The results of routine laboratory tests, urine analyses, and electrocardiograms were not modified by losartan. The values of blood pressure and heart rate after the single and multiple dosing of losartan are summarized in Tables 3 and 4, respectively. In the single-dose study, changes in blood pressure and heart rate induced by the dosing were calculated as the difference between pre-drug and post-drug levels for each subject at each dose and at each time. The peak hypotensive effect was reached 6 h after intake of the highest (200 mg) dose of losartan, and the changes in systolic blood pressure were 10–12 mm Hg of the pre-drug levels. In the multiple-dose study, when compared with placebo, no clear effect was observed with the dose of 100 mg. The changes in PRA and plasma concentrations of AII after single dosing are presented in Figure 3. Both PRA and AII levels showed a marked increase 6 h post-dosing at all dose levels. With the dose of 200 mg, even 24 h post-dosing, a definite effect was still present. Figure 4 depicts the PRA and plasma AII concentrations measured at the beginning and the end of repeated dosing of losartan or placebo. Both PRA and AII showed a marked increase 6 h post-dosing. On day 7, pre-drug levels of PRA as well as plasma AII were greater than those obtained for placebo, while the pre-drug levels obtained on day 1 were all very similar. The mean aldosterone levels decreased slightly after single and multiple doses of losartan, but a similar decrease was also observed after placebo.

Table 3 Supine systolic blood pressure and heart rate after single oral administration of different doses of losartan

	Dose (mg)			
Time (h)	*25*	*50*	*100*	*200*
Blood pressure (mm Hg)				
Pre	112 ± 7	115 ± 7	114 ± 12	110 ± 7
0.5	112 ± 3	113 ± 8	114 ± 11	107 ± 5
1	112 ± 5	112 ± 12	113 ± 15	105 ± 6
2	109 ± 6	112 ± 2	113 ± 12	108 ± 5
3	104 ± 3*	107 ± 8*	110 ± 11	103 ± 3
4	109 ± 5	110 ± 7*	110 ± 12	103 ± 5
6	110 ± 7	106 ± 7*	108 ± 12	98 ± 6*
8	108 ± 5	106 ± 8*	107 ± 10*	100 ± 6
10	112 ± 6	109 ± 6*	111 ± 11	101 ± 2*
12	112 ± 6	108 ± 5*	112 ± 12	108 ± 6
14	113 ± 6	109 ± 7	112 ± 10	103 ± 6
24	112 ± 5	109 ± 7*	106 ± 9*	104 ± 3
30	118 ± 9	110 ± 9	110 ± 11	109 ± 7
Heart rate (beats min^{-1})				
Pre	57 ± 7	58 ± 10	58 ± 6	62 ± 9
0.5	58 ± 5	57 ± 9	60 ± 6*	61 ± 7
1	58 ± 6	56 ± 7	59 ± 5	62 ± 7
2	58 ± 8	56 ± 7	59 ± 6	61 ± 9
3	57 ± 8	54 ± 8	57 ± 4	60 ± 7
4	57 ± 6	55 ± 8	59 ± 7	61 ± 9
6	66 ± 6*	63 ± 9*	67 ± 4*	62 ± 6
8	64 ± 4*	58 ± 6	66 ± 4*	63 ± 9
10	63 ± 5*	58 ± 8	62 ± 5	65 ± 10
12	65 ± 6*	63 ± 8*	69 ± 8*	67 ± 8
14	66 ± 6*	63 ± 6	65 ± 5*	65 ± 11
24	57 ± 6	56 ± 8	62 ± 6*	58 ± 9
30	69 ± 4*	66 ± 8*	71 ± 3*	68 ± 10

Each value represents the mean ± s.d. of six subjects.
*$P < 0.05$.

Table 4 Supine blood pressure and heart rate before and during 7 days of administration of losartan or placebo

Day of and time after dosing (day/h)	*Losartan* (n = 6)		*Placebo* (n = 3)	
	Blood pressure (mm Hg)	*Heart rate (beats min^{-1})*	*Blood pressure (mm Hg)*	*Heart rate (beats min^{-1})*
1/pre	117/71 ± 18/16	60 ± 7	115/70 ± 4/3	54 ± 8
1/6	107/57 ± 17/14	66 ± 7	109/59 ± 7/3	58 ± 5
2/0	111/62 ± 13/12	70 ± 9	110/59 ± 4/1	64 ± 5
2/6	105/57 ± 12/11	73 ± 9	111/60 ± 12/9	61 ± 6
3/0	114/64 ± 14/9	70 ± 7	115/62 ± 5/5	63 ± 3
3/6	108/59 ± 9/9	74 ± 7*	112/60 ± 3/1	63 ± 3
4/0	113/64 ± 12/9	74 ± 5	117/64 ± 1/1	67 ± 4
4/6	112/63 ± 17/19	73 ± 8	119/63 ± 7/3	67 ± 1
5/0	111/63 ± 13/14	72 ± 7	112/61 ± 16/9	67 ± 8
5/6	108/60 ± 17/16	74 ± 6*	111/61 ± 7/5	65 ± 1
6/0	114/63 ± 16/10	70 ± 5	116/91 ± 6/5	64 ± 4
6/6	110/59 ± 16/14	72 ± 6*	113/60 ± 10/4	59 ± 2
7/0	107/63 ± 19/16	61 ± 5	108/64 ± 11/5	56 ± 5
7/6	103/54 ± 14/11	65 ± 4	114/61 ± 14/10	59 ± 6
8/0	107/62 ± 15/12	64 ± 6	107/61 ± 8/4	57 ± 4
8/6	108/60 ± 14/12	70 ± 6	107/57 ± 8/5	65 ± 4
9/0	112/66 ± 16/14	66 ± 8	109/63 ± 2/3	59 ± 7

Each value represents the mean ± s.d.
*$P < 0.05$.

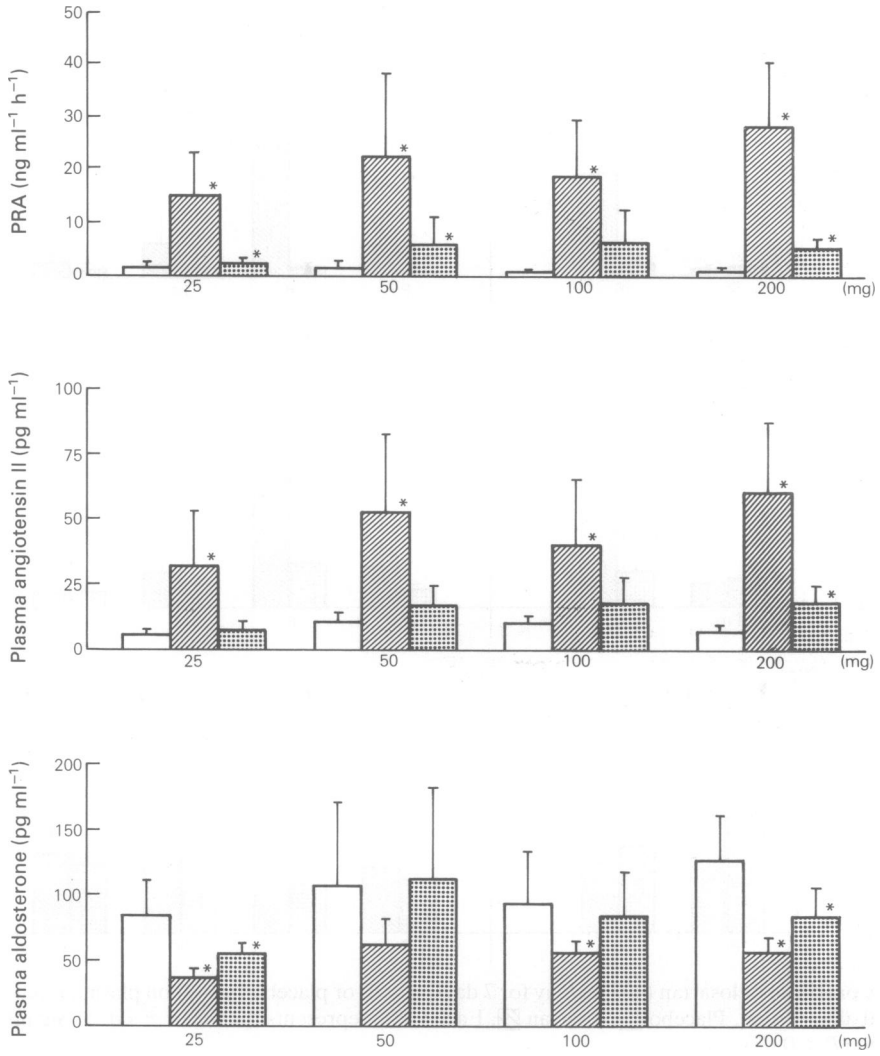

Figure 3 Effect of different doses of single oral losartan on plasma renin activity (PRA), plasma concentrations of AII and aldosterone ($n = 6$). Pre drug □; 6 h post-dosing ▨; 24 h post-dosing ▩. Each value represents the mean ± s.d. of six subjects. *: Significant compared with the value of pre-drug ($P < 0.05$).

Discussion

The study to evaluate the effect of dose on pharmacokinetics after single oral administration of losartan demonstrated that the plasma concentrations of the parent drug and its major active metabolite, E-3174, increased with increasing dose over the range of 25 to 200 mg and that the terminal half-lives for losartan and E-3174 were independent of dose. No saturation of the absorption at doses of up to 200 mg occurred. The plasma concentrations of E-3174 were higher than those of losartan at all dose levels. The values of C_{max} and AUC0-∞ for E-3174 were also higher and the value of $t_{1/2,z}$ was longer than those for parent drug. At all dose levels, the plasma concentrations of losartan at 10 h post-dosing were under the quantitation limits of the h.p.l.c. assay. On the other hand, E-3174 was detectable in plasma even at 24 h post-dosing.

After multiple dosing for 7 days, in the case of losartan, a statistical difference ($P < 0.05$) was found when comparing the value of AUC0-∞ on day 1 with that of AUC0-24 on day 7. However, the accumulation ratio (AUC0-24/AUC0-24, day 7/day 1) for losartan was about 0.8, and each value of C_{max} and AUC0-24 for either losartan or E-3174 was not significantly different between day 1 and day 7. The plasma levels of losartan and E-3174 at 24 h after every dosing did not increase, and those urinary excretions within every 24 h post-dosing did not change significantly during and after multiple dosing. These results suggest that neither accumulation of the drug in plasma nor metabolic changes of the drug occurred.

To define clearly the biochemical efficacy, PRA, plasma levels of AII and aldosterone were measured. PRA and plasma AII levels increased markedly at all dose levels. With the highest dose, 200 mg, even 24 h post-dosing a definite effect was still present. These data demonstrate that losartan is a potent orally active AII receptor antagonist with a relatively long duration of action. Wong *et al.* (1990d) reported that the blocking action of E-3174 on the AII receptor is about 41 times more potent than the parent compound, losartan, based on the apparent pA_2 values in rabbit aorta, and the ED_{30} value for the hypotensive activity of E-3174 on hypertensive rats is about 1/20 of that of losartan. Therefore, the long-lasting effect on PRA and plasma AII after oral

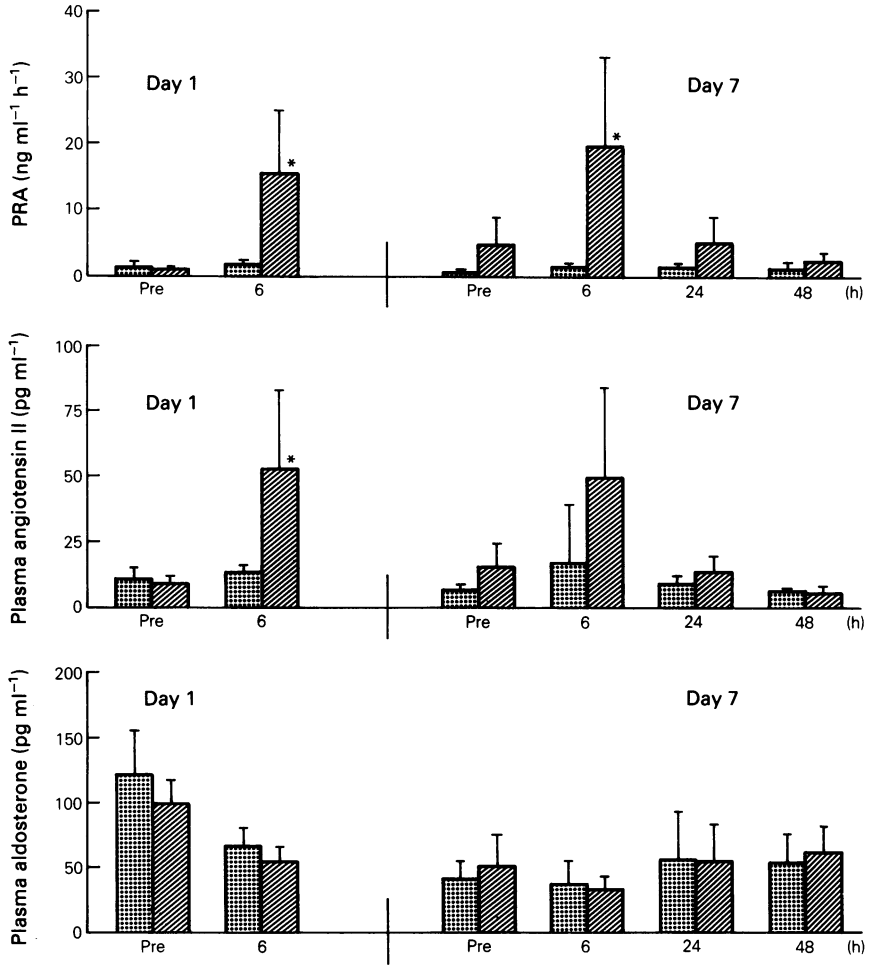

Figure 4 Effect of daily oral dose of losartan (100 mg/day for 7 days, $n = 6$) or placebo ($n = 3$) on plasma renin activity (PRA), plasma levels of AII and aldosterone. Placebo ▒; losartan ▨. Each value represents the mean ± s.d. *: Significant compared with the value of placebo ($P < 0.05$).

administration of losartan may have resulted from the retention of plasma levels of E-3174.

After multiple dosing, the increase in PRA was more pronounced on day 7 of drug administration than on day 1. This tendency is in good agreement with the results reported by Christen *et al.* (1991a,b). On day 7, pre-drug levels of PRA as well as plasma AII increased as compared with the pre-drug levels obtained for placebo. The long duration of the blocking effect of losartan is also reflected in the PRA and plasma AII. Plasma aldosterone levels were slightly reduced after dosing, but a similar decrease was also observed after placebo. The effect of losartan on PRA is much greater than on aldosterone. The reason for this different efficacy is not clearly understood.

Losartan had a slight decrease of resting blood pressure and no effect on pulse rate after single and multiple dosing. Since the drug was administered only to normal human volunteers, it cannot rule out the existence of anti-hypertensive effect of losartan. The findings obtained with losartan in hypertensive animals and the earlier observations made with saralasin suggest strongly that losartan will have therapeutic utility for treatment of hypertension.

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